BRIEFER ARTICLES

PARAFFIN SOLVENTS IN HISTOLOGICAL WORK

In the last few years there have been described in the BOTANICAL GAZETTE two ways of improving the common method of replacing xylol with paraffin in histological work. The alleged defect in the old method lies in the fact that the paraffin sinks to the bottom of the vessel and immediately surrounds the specimens with a concentrated solution. LAND'S[1] remedy for this is to support the paraffin near the surface of the xylol in a basket of wire gauze; GOODSPEED[2] molds the paraffin into a lump that will fit the containing vessel and rest on top of the xylol. Both of these methods have been found good for accomplishing the end desired, and a third may be of interest.

The paraffin to be used can, by aëration, be rendered capable of floating in xylol, the method of preparation being similar to that employed in giving buoyancy to some kinds of soap. While the paraffin is at a temperature only a degree or two above that required for melting, a current of cold air is bubbled through it, causing it to harden as a frothy mass. This mass is then kneaded to secure finer grain and more even distribution of the air bubbles. Only a small amount of air is needed to produce the proper buoyancy, and it can be supplied from any one of numerous sources and cooled by passing through a condenser. Although the method may seem troublesome, it is really not so; a large amount of paraffin may be prepared at one time, and nothing further is necessary to get the desired results.

Although this method of holding the paraffin at the top of the xylol has been found successful and easy of manipulation, I do not employ it as a rule, because I have not yet seen the defect of the old method of adding the paraffin when xylol is used, and chloroform has been found perfectly satisfactory in all cases. If, when xylol is used, the necessary amount of paraffin be added in a finely divided condition in 5 or 6 portions, and diffusion aided by gentle agitation, a

[1] LAND, W. J. G., Microchemical methods, an improved method of replacing the paraffin solvent with paraffin. BOT. GAZ. 59:397. 1915.

[2] GOODSPEED, T. H., Method of replacing paraffin solvent with paraffin. BOT. GAZ. 66:381–382. 1918.

sufficiently gradual change is secured. When the paraffin is to be supported at the surface of the solvent, by buoyancy or other means, it is better to add it in a round lump, because in this case slow diffusion is desirable; but, when it is allowed to sink in the xylol, it should be added in small fragments, since quick diffusion is desirable.

Although in many laboratories xylol has taken the place of chloroform in work of this kind, the latter is still used by many technicians because of certain advantages that it possesses over xylol. The object in using either is not, as is often stated, to "clear" the specimen, but to provide a medium for the introduction of the paraffin. Actual "clearing," that is, the production of transparency, is of no benefit at all in this stage of the process; in fact, it proves a disadvantage that can be overcome only by staining *in toto* when only a few sections from a long ribbon are to be selected for mounting. Chloroform has much less tendency to produce transparency than has xylol. Moreover, as DUGGAR[3] has stated, a peculiarly undesirable optical effect is produced permanently by xylol in some tissues. The use of chloroform makes unnecessary, of course, any device for holding the paraffin at the top, if this is thought necessary.

The higher specific density of chloroform, which LAND[4] has mentioned as a factor to be considered, is aside from the question; it has long been known that permeability and osmotic pressure, the things which determine shrinkage, are not, as was once supposed, determined by density when pure substances are being compared. Moreover, neither chloroform nor xylol could "plasmolyze" a cell, as LAND states; at this stage of the process a cell is not subject to such life phenomena as plasmolysis.

The item of expense, which has caused the use of chloroform to be discontinued in many places, and which is of special significance just now, can be lessened by the easy and inexpensive recovery of about one-half of the reagent used in the ordinary process. The waste chloroform, containing alcohol or other impurities, is thrown out of solution with a large quantity of water, dried with calcium chloride, which removes both alcohol and water, and distilled, the boiling point being observed as a check on the purity of the product.—PAUL WEATHERWAX, *Indiana University.*

[3] DUGGAR, B. M., Fungous diseases of plants. New York. 1909. p. 49.

[4] In a note added to an article by MOTTIER, BOT. GAZ. **61**:253. 1916.